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Bis- and Trisuracil Nucleosides with Nucleobases Anchored to 11-, 12-, and 16-Membered Macrocyclic Scaffolds: Synthesis and Aggregation Study

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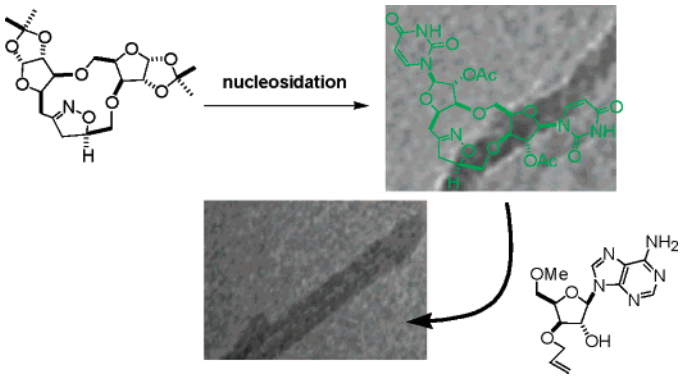

Bis- and trisuracil nucleosides, in which the nucleobases are anchored to isoxazoline ring-fused 11-, 12-, and 16-membered macrooxacycles, were synthesized by nucleosidation of 1,2-isopropylidenefuranose ring-fused macrocycles. The nucleosides exhibited spherical and fiber-like morphologies in water. In one case, the morphology was significantly altered by complexation with an adenine nucleoside via complementary base pairing.

Interaction of nucleobases with themselves and other molecules has a profound role in biological systems. Supramolecular assemblies made out of derivatives of nucleobases have been examined by various techniques, and different motifs arising out of hydrogen bonding, stacking, and hydrophobic interactions have been proposed.[1] It is well-established that molecular systems such as lipids or other amphiphilic molecules containing both polar and apolar units such as oligomethylene chains lead to ordered structures in water primarily due to the involvement of both hydrogen bonding and hydrophobic interactions. Supramolecular complexes arising out of complementary base pairing between non-nucleoside molecules such as melamine and cyanuric acid derivatives have been extensively studied.[2] Interesting structural motifs have been proposed for supramolecular complexes from guanine and cytosine and, in fewer cases, from adenine and uracil (or thymine) derivatives. Self-assembly of lipophilic monoguanosine derivatives were reported to give rise to ribbon-like helical aggregates,[3a] whereas phospholipid−mononucleoside conjugates afforded helical aggregates of diverse morphologies in aqueous solutions.[3b] In this context, molecules containing dinucleoside units offer a unique opportunity for making supramolecular assemblies by virtue of their ability to form polymeric structures. Bolaamphiphiles

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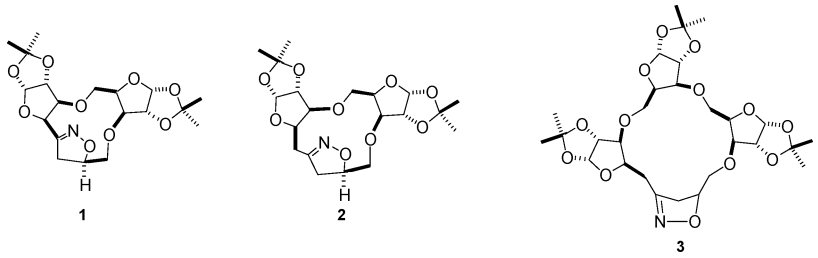

FIGURE 1. The 11-, 12-, and 16-membered oxacycles fused to isoxazoline and 1,2-isopropylidenefuranose rings.

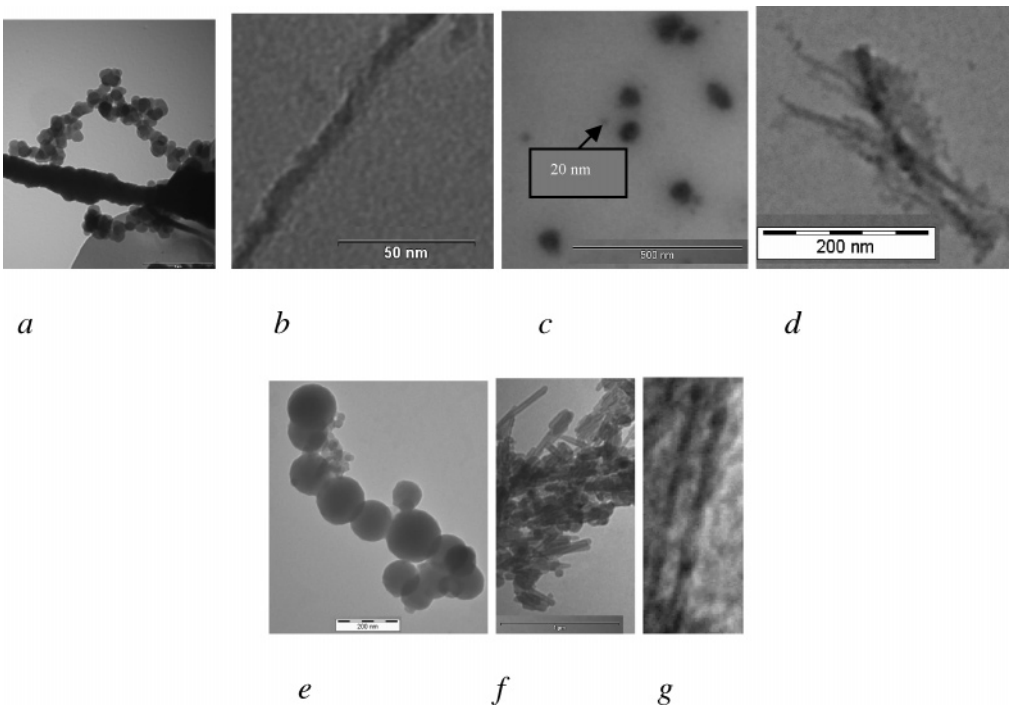

FIGURE 2. TEM (bright field; unstained; aq solution) pictures of (*a*) spherical aggregates from **6** at 10^{-4} M; a column of stacked spheres can be seen at the bottom (scale bar = 1 μm, 60 kV); (*b*) fiber from **7** at 10^{-4} M (scale bar = 50 nm, 60 kV); (*c*) spherical aggregates from **6** at 10^{-5} M; (*d*) fiber from **7** at 10^{-5} M; (*e*) spherical aggregates from a 1:1 mixture of **6** and **12**; (*f*) rods from a mixture of **7** and **12** (scale bar = 1000 nm, 60 kV); (*g*) nanotube-like structure in *f* at 120 kV (width ~28 nm).

containing 3′-phosphorylated or free nucleobases as head groups tethered by long oligomethylene spacers underwent aggregation in water, giving rise to gel, fibers, and helical ropes, either by themselves or in combination with complementary oligonucleotides.[4] The self-assembly of a monomer prepared by appending adenine and thymine rings at both ends of a crown ether unit led to the formation of a "molecular box" via H-bonding as evidenced by NMR spectroscopic and other physical studies.[5] A trimeric complex has been realized from an olefin-tethered guanosine−cytosine dinucleotide[6] and, in an extreme case, polyether-tethered diadenine derivatives self-assembled to give fibers and transparent films.[7] We were curious to know how bisuracil derivatives, in which the nucleobases are anchored to rigid medium-ring scaffolds lacking hydrophobic tethers, would behave as aggregating units in water by taking advantage of H-bonding and stacking processes as primary interactions. Another objective was to study whether these nucleosides would bind to an adenine derivative via a specific mode. Recently, we reported the synthesis of the first examples of 3,5′-ether-linked pseudooligosaccharides as well as the cycloaddition of nitrile oxides generated from these compounds, leading to the formation of unique macrocycles such as **1**, **2**, and **3** (Figure 1).[8] An interesting feature of these macrocycles is that **1** and **2** incorporate isoxazoline ring-fused 11- and 12-membered macrocycles, whereas **3** is made up of a similarly fused 16-membered ring. The other feature of these chiral macrocycles is the presence of 1,2-isopropylidene-protected furanose rings, which are amenable to various transformations resulting in multiple functionalities.[8] It was envisioned that these macrocycles could also be subjected to nucleosidation reaction of the furanose rings, thereby providing examples of structurally unique

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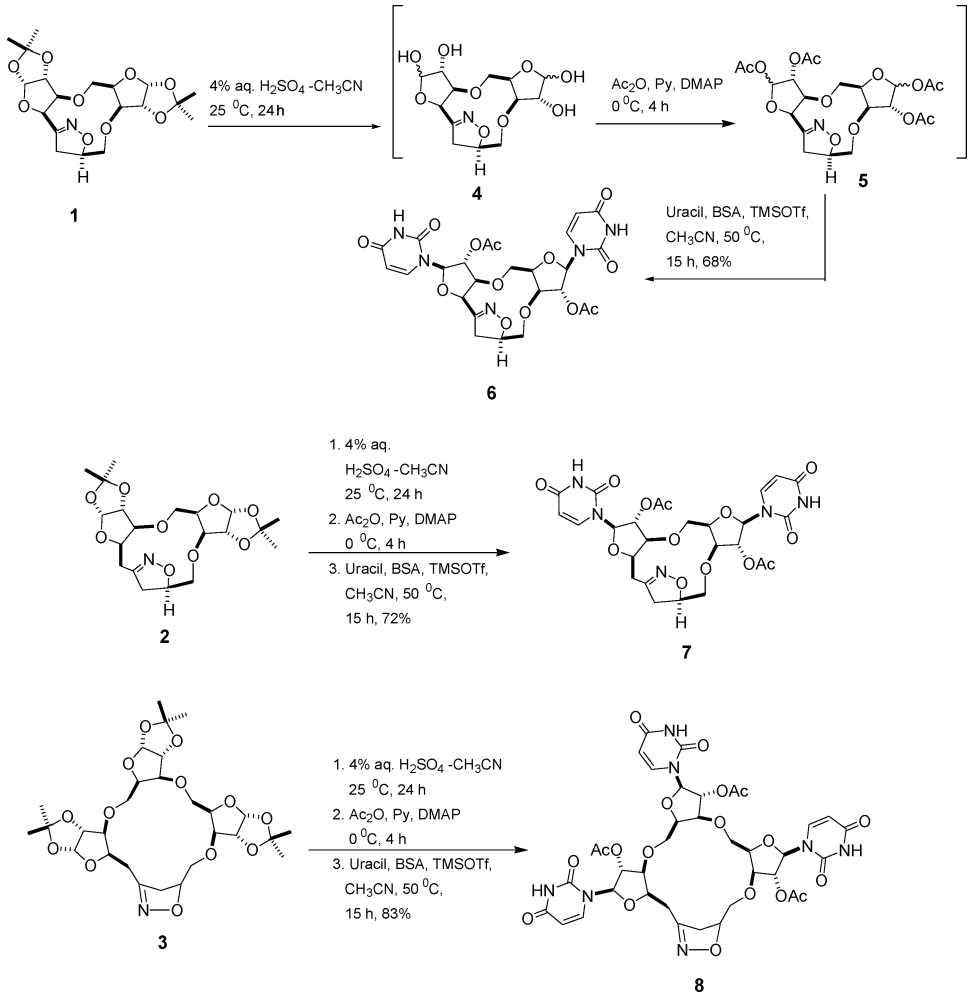
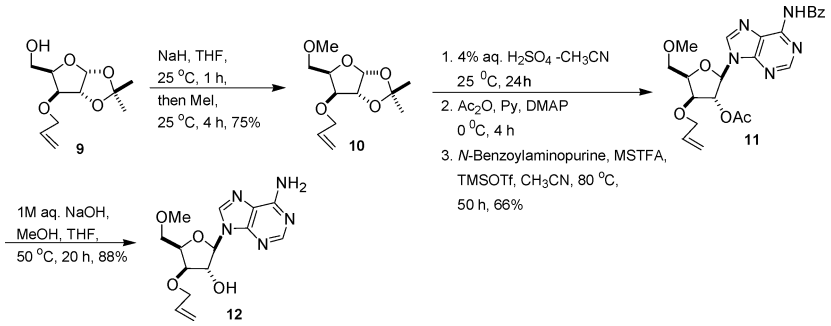
SCHEME 1. Synthesis of Cyclic Uracil Nucleosides 6, 7, and 8



SCHEME 2. Synthesis of the Adenine Derivative 12



constrained cyclic nucleosides. We report herein the synthesis of nucleosides from **1**, **2**, and **3** and some preliminary results on their aggregation behavior as well as their ability to bind to complementary adenine nucleoside.

The general strategy for the synthesis of the cyclic nucleosides described in this work is illustrated in Scheme 1 by the conversion of **1** to the nucleoside **6**. Removal of the isopropylidene protection was achieved by treatment with 4% aqueous $H_2SO_4-CH_3CN$ for 24 h. The resulting tetrahydroxy intermediate **4**, being a mixture of four possible diastereomers due to the presence of α and β anomers of the two furanose rings, was carried to the next step without further purification. Acetylation of **4** led to the formation of the anomeric mixture of the tetraacetate **5**, which was also directly used for the subsequent nucleosidation reaction. Nucleosidation of **5** was performed by applying the Vorbrüggen method,[5,9] and treatment of **5** with uracil in the presence of *N*,*O*-bis-trimethylsilyl acetamide (BSA) and trimethylsilyl triflate at 50 °C afforded the bisuracil nucleoside **6** in 68% overall yield. The skeletal nature of the core macrocycle remained unaffected by the synthetic operations, and the incorporation of the uracil units was evident from NMR, mass, and IR spectral analyses. Similarly, the macrocycles **2** and **3** were subjected to the above-mentioned synthetic protocol for nucleosidation, and the uracil derivatives **7** and **8** were obtained in 72 and 83% yields, respectively (Scheme 1).

In order to study the complexation with a complementary

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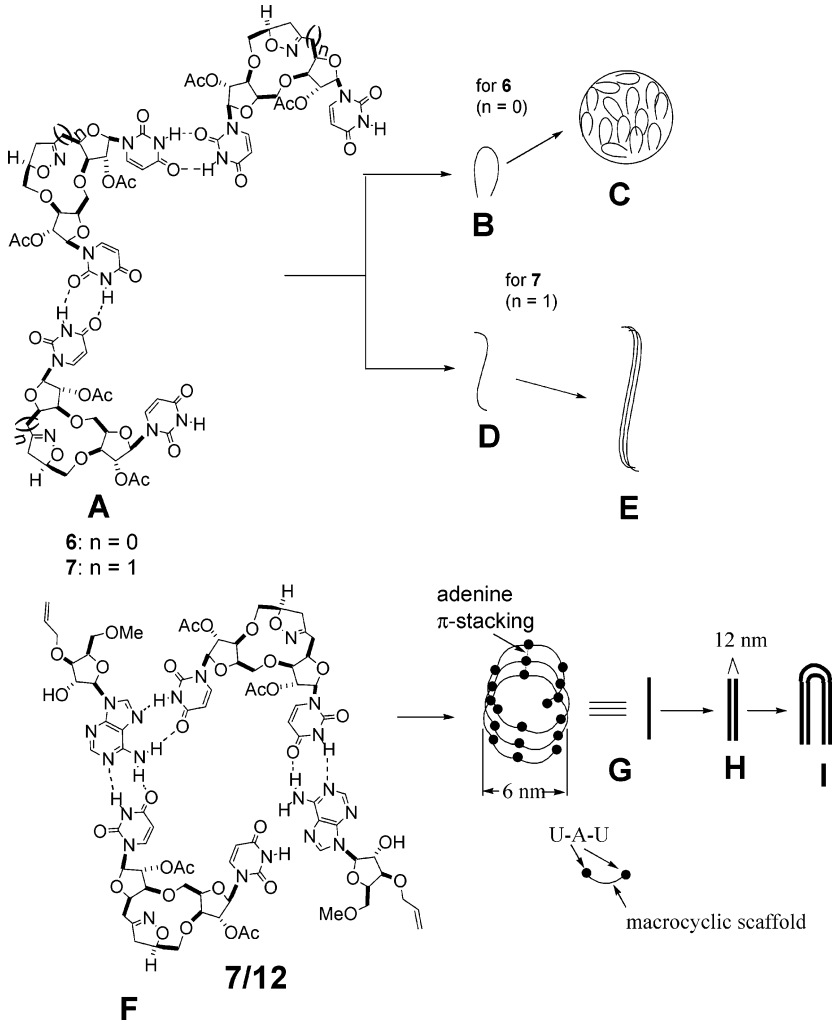
SCHEME 3. Proposed Models for the Aggregation of 6, 7, and Their Mixtures with 12



nucleobase derivative, the adenine nucleoside **12** was synthesized from the known glucose derivative **9** via **10** and the *N*-benzoyladenosine derivative **11** according to Scheme 2.[10]

Recently, a bisuracil derivative in which the uracil rings are joined by an oligomethylene tether has been found to undergo aggregation in water, leading to the formation of helical ropes.[4a] The uracil rings in **6** and **7** are anchored to rigid scaffolds consisting of medium rings and several oxygen and nitrogen atoms. These nucleosides do not contain any hydrophobic groups, which were conventionally present in earlier studied nucleoside derivatives forming regular morphologies.[3,4] We were curious to know whether these nucleosides having the aforementioned structural features were capable of undergoing aggregation in water, leading to the formation of regular morphologies. A sample prepared by sonicating a 10^{-4} M aqueous solution of **6** at 25 °C showed, after 48 h, clusters of spherical aggregates (sphere diameter ~150 nm), which stacked to form columnar structures shown in Figure 2a. Formation of the spherical aggregates was evident even after 3 h. The possibility of an alternative disk nature of the aggregates was ruled out by tilting experiments during microscopy. Although detailed nature of the aggregates has not been investigated, the spherical shapes prevailed in different micrographs of **6** in water.

Unlike **6**, TEM of a 10^{-4} M aqueous solution of **7** revealed long flexible fibers of ~7 nm diameter (Figure 2b). Helical fibers have been observed for bisuracil derivatives, in which the uracil rings are tethered by long oligomethylene chains.[4] In contrast to **6** and **7**, TEM of **8** revealed only bundles of fibers (Supporting Information) in 10^{-4} M aqueous solution. In this case, the presence of three uracil rings presumably led to extensive networking. In order to determine whether the nanostructures of these nucleosides depended on concentration, TEM images of **6**, **7**, and **8** were obtained in 10^{-5} M aqueous solutions. TEM of **6** exhibited globular structures similar to those observed earlier for 10^{-4} M solutions, but smaller (~20 nm) globular aggregates (Figure 2c) could be observed at this concentration. This indicated that these smaller structures further aggregated to form larger globular morphologies. The fiber morphology of **7** persisted in the 10^{-5} M solutions. Although long extended fibers could not be found, short lengths of fibers similar in width as those observed earlier were present (Figure 2d). Unlike **6** and **7**, the morphology of **8** changed at 10^{-5} M concentration, and globular aggregates (Supporting Information) were obtained.

Purine bases are known to have higher π-stacking ability than pyrimidine bases, and it was of interest to study whether addition of the adenosine derivative **12** would alter the aggregation pattern of **6** and **7** by complexation via complementary base pairing as well as π-stacking interactions. With this end in view,

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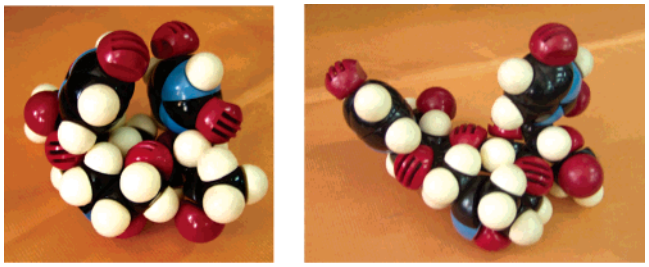

a *b*

FIGURE 3. CPK molecular models of **6** (*a*) and **7** (*b*).

a 1:1 mixture of **6** and **12** in water was subjected to TEM. Clusters of spheres (sphere diameter ~75−160 nm) similar to those observed for **6** alone were found (Figure 2e). This indicated that addition of **12** had no significant effect on the aggregation of **6**. A distinctly noticeable change in morphology was observed in the case of **7**, and TEM of an aqueous solution of **7** and **12** (**7**:**12**, 1:1) exhibited nanotube-like aggregates after 48 h (Figure 2f,g). As a control, TEM of an aqueous solution of **12** showed only large lamellar aggregates with no regular structure (Supporting Information).

One important feature of the nucleosides **6** and **7** is that they do not contain any hydrophobic group such as long oligomethylene chains. Also, uracil rings are poorly π-stacking, so it is logical to assume that the aggregation of **6** and **7** in water is mainly due to H-bonding. Although it is difficult to explain why spherical aggregates are observed from **6** unlike fibrillar aggregates seen for **7**, a tentative scheme for the hierarchical organization of the aggregates is presented in Scheme 3. It is proposed that both **6** and **7** form polymeric H-bonded structures **A**. Small fibrillar aggregates **B** of **6** are formed as primary structures, which further aggregate, leading to the spherical morphologies **C**. In the case of **7**, extended fibrillar aggregates such as **D** are formed, which further aggregate, giving rise to fiber morphologies **E**. CPK molecular models of **6** and **7** indicated that the former adopts a folded conformation, in which the two uracil rings are proximate to each other, as shown in Figure 3a. The isoxazoline-fused 11-membered scaffold in **6** makes it a rigid structure. Unlike **6**, the homologous 12-membered scaffold in **7** is slightly more flexible, so **7** can adopt a more relaxed conformation, as shown in Figure 3b. It is possible that **12** cannot bind to **6** favorably due to its folded conformation, so no drastic change in morphology was observed for **6**. In contrast, it is easier for **12** to bind simultaneously to two uracil rings belonging to two different molecules of **7**, as shown in **F** due to the more relaxed conformation of **7**. This leads to the formation of a helical assembly such as **G** (width ~6 nm, CPK model) as primary structures. The rigidity of **G** may be ascribed to π-stacking interaction between the adenine rings. Further aggregation of **G** gives rise to fibrillar structures **H** (width ~12 nm), which undergo bending to form the nanotube-like structures **I**. More detailed study of these aggregates is expected to give a clearer picture of the aggregation behavior of these unusual nucleosides.

In conclusion, this work established that the dinucleosides **6** and **7**, despite lacking long hydrophobic units, formed reproducible aggregates of regular morphologies in water, and in one case, the morphology was significantly altered by complementary base pairing. The study is expected to be useful for the understanding of similar phenomena in biological systems.

Experimental Section

General Procedure for the Nucleosidation of the Macrocycles 1, 2, and 3. The macrocycles **1**, **2**, and **3** were synthesized according to the reported method.[8] The general procedure for the synthesis of the nucleosides **6**, **7**, and **8** is illustrated for the bisuracil derivative **6**.

A stock solution was prepared by mixing CH_3CN, water, and concd H_2SO_4 in volumetric ratio of 18:6:1. A mixture of **1** (0.15 g, 0.036 mmol) in this solution (10 mL) was stirred at 25 °C for 24 h. The solution was neutralized by adding solid $CaCO_3$, and the mixture was filtered. The residue was washed with acetonitrile, and the combined washings were concentrated under reduced pressure and dried under vacuum. To a solution of the resulting deprotected compound **4** in pyridine (10 mL) were added acetic anhydride (0.16 mL, 1.80 mmol) and a catalytic amount of DMAP at 0 °C. The mixture was stirred at 25 °C for 4 h. Excess Ac_2O was destroyed by adding water, and the resulting AcOH was removed by azeotropic distillation with toluene. The residue was chromatographed (EtOAc/petroleum ether, 3:2) to give **5** as a syrupy liquid (0.15 g), which was dried under vacuum. To a solution of this material (0.15 g, 0.3 mmol) in CH_3CN (10 mL) containing uracil (0.13 g, 1.20 mmol) was added with stirring BSA (0.74 mL, 2.98 mmol), and the mixture was heated at reflux for 1 h. Then TMSOTf (0.2 mL, 1.03 mmol) was added at 0 °C, and the mixture was heated at 50 °C for 15 h. The reaction was quenched with cold saturated $NaHCO_3$ solution. After removal of solvent, the residue was extracted with ethyl acetate, washed with brine, dried, and concentrated to afford a sticky liquid, which was chromatographed ($CHCl_3$/MeOH, 49:1) to give **6** (0.15 g, 68%) as a white solid: mp 180−182 °C; $[\alpha]_D^{25}$ +4.2 (*c* 0.55, $CHCl_3$); IR (KBr) 1694, 1746, 3465 cm^{-1}; MS (FAB) *m*/*z* 628 (M + Na), 606 (M + H); ^1H NMR δ 2.12 (s, 3H), 2.14 (s, 3H), 3.11 (dd, J = 11.0, 17.1 Hz, 1H), 3.28 (dd, J = 3.0, 17.0 Hz, 1H), 3.59 (d, J = 11.9 Hz, 1H), 3.73 (d, J = 12.2 Hz, 1H), 3.98 (d, J = 10.3 Hz, 1H), 4.08 (d, J = 2.4 Hz, 1H), 4.18−4.27 (m, 3H), 4.72 (d, J = 8.8 Hz, 1H), 5.08 (s, 1H), 5.26 (d, J = 6.2 Hz, 1H), 5.56 (d, J = 5.3 Hz, 1H), 5.69 (d, J = 3.1 Hz, 1H), 5.80 (d, J = 8.0 Hz, 1H), 5.99 (s, 1H), 6.18 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.5 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H), 9.80 (br s, 1H), 10.40 (br s, 1H); ^{13}C NMR δ 20.70 (CH_3), 20.73 (CH_3), 37.1 (CH_2), 68.7 (CH_2), 72.1 (CH_2), 76.4 (CH), 78.6 (CH), 79.1 (CH), 79.6 (CH), 80.3 (CH), 83.2 (CH), 84.5 (CH), 88.5 (CH), 90.8 (CH), 101.9 (CH), 103.4 (CH), 141.0 (CH), 141.7 (CH), 149.9 (q), 150.3 (q), 157.0 (q), 163.9 (q), 164.6 (q), 169.5 (q), 170.0 (q). Anal. Calcd for $C_{25}H_{27}N_5O_{13}$: C, 49.59; H, 4.49; N, 11.57. Found: C, 49.30; H, 4.69; N, 11.30.

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Supporting Information Available: ^1H and ^{13}C NMR spectra, TEM of **6**, **7**, **8**, **6**/**12**, **7**/**12**, and **12**. Characterization of **7** and **8** and experimental procedures for **10**, **11**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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